bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Mitochondrial pyruvate transport assay

RA Robinson Arce-Molina LB L Felipe Barros AS Alejandro San Martín

Updated date: Jul 6, 2021

An abbreviated version of this protocol was published in eLIFE in Mar 2020

A highly responsive pyruvate sensor reveals pathway-regulatory role of the mitochondrial pyruvate carrier MPC

DOI: 10.7554/eLife.53917

Detailed protocol

Simplest is to measure the rate of fluorescence change in response to a pyruvate pulse (typically 3 mM). For accurate rate determination is necessary to obtain several experimental points during the linear phase of accumulation, so a smaller pyruvate pulse (e.g. 1 mM) should be selected when uptake is too fast. In mammalian cells that express abundant monocarboxylate transporters (MCTs) at their cell surface, the speed of mitochondrial pyruvate accumulation is usually limited by mitochondrial pyruvate carrier (MPC) permeability and therefore it is possible, and less invasive, to study the MPC using intact cells. In the absence of pyruvate in the perfusate the concentration of pyruvate within mitochondria (20-40 uM) is well below the K_D of mitoPyroniSF (480 uM), so the protocol may be applied to cells fed with glucose, lactate or any other substrate, except pyruvate. NB: if MPC permeability happens to be higher than surface permeability then cells will need to be permeabilized to study the MPC. The relative speed of cellular versus mitochondrial pyruvate uptake may be checked by comparing the rates of fluorescence increase of PyronicSF expressed in cytosol versus mitochondria.

Standard Protocol:
1.- Mount cells in a fluorescence microscope and superfuse them at > 2 ml min with an appropriate buffer devoid of pyruvate (see examples in the article). To obtain accurate rates of accumulation it is important that the turnover time of the perfusion chamber is not more than 15 sec so that the buffer is quickly exchanged. Imaging cells that are close to the perfusion inlet speeds up local perfusate turnover.
2.- Apply the pyruvate pulse (1-10 mM) for 2 minutes (similar to Figure 2C in the article). The uptake rate is the slope of the linear phase of signal increase (usually the first 30-60 seconds of the pyruvate pulse). Adjust sampling frequency so at least 6 points are obtained during the linear phase (0.1 - 0.5 Hz).
3. End of the experiment.
For some purposes it may be useful to obtain absolute rates (mass/time) of pyruvate uptake. This may be achieved using a one-point calibration based on the minimum fluorescence (F_{MIN}). F_{MIN} is obtained at the end of the experiment by exposing cells to 10 mM lactate immediately after the pyruvate pulse. Lactate enters via MCTs and trans-accelerates any residual pyruvate out of the cells, thus emptying the sensor. Fluorescence is than converted to pyruvate concentration using F_{MIN} and the values of K_D and ΔF_{MAX} reported in the article.
4.- Perfuse with 10 mM lactate for 5 minutes to reach F_{MIN} (similar to figure 3A in the paper).

NOTES:

- Proper extrusion of pyruvate using trans-acceleration with lactate requires MCT expression at the plasma membrane, which is the case for most mammalian cells.
- Sensor fluorescence is saturable and therefore does not increase linearly with pyruvate concentration. Therefore, slopes at different parts of the curve should not be compared. In order to compare rates, slopes must be extracted at the same fluorescence level (F- F_{MIN}).
- To inhibit the MPC, cells are preincubated with 10 uM UK-5099 for 5 min before the pyruvate pulse. In our experience, the effect of UK-5099 is not fully reversible.

Is a normal fluorescence microscope enough to detect the changes?
Answer: a standard fluorescence microscope with an FITC filter is enough to image whole cells. A high numerical aperture objective will collect more light and increase the signal to noise ratio. Imaging discrete mitochondria may require confocal microscopy.

Also, how would you distinguish between pyruvate concentrations in the mitochondria and in cytosol?
Answer: If the sensor if properly targeted to mitochondria there will be negligible cytosolic fluorescence. The easiest is to discard cells in which there is a sizable cytosolic fluorescence (leak). Alternatively, sensor targeting may be optimized by tweaking the expression protocol (DNA/virus concentration, time of transfection/transduction, etc). Suboptimal mitochondrial targeting may be fixed by increasing the number of targeting sequences in the construct.

Do I need to permeabilize my cells in order to track pyruvate transport to the mitochondria?
The approaches described in the PyronicSF paper do not require permeabilization. Imaging permeabillized cells is possible but the procedure is invasive and may perturb the functional properties of the carrier or its regulation.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Arce-Molina, R. , Barros, L. and San Martín, A. (2021). Mitochondrial pyruvate transport assay. Bio-protocol Preprint. bio-protocol.org/prep1263.
2. Arce-Molina, R., Cortés-Molina, F., Sandoval, P. Y., Galaz, A., Alegría, K., Schirmeier, S., Barros, L. F. and San Martín, A.(2020). A highly responsive pyruvate sensor reveals pathway-regulatory role of the mitochondrial pyruvate carrier MPC. eLIFE. DOI: 10.7554/eLife.53917